Quantitative Analysis for Proteomic Profiling of Signal Transduction Pathways in Ovarian Tumors

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Profiling the activity of signaling pathways in the context of the disease process can highlight disease-related alterations that represent the patient-tailored drug targets of the future. We are working to characterize the complex rewiring of protein signaling networks that occurs during the progression of invasive ovarian cancer. Epithelial cell lysates were generated from a number of primary ovarian tumors and spotted in serial dilution curves onto nitrocellulose-coated microscope slides. Individual arrays were probed with phosphospecific antibodies to assess the levels and activation status of a number of proteins that act as sensors and amplifiers in a variety of cell signaling pathways. Spot intensities were calculated using P-SCAN, a general purpose quantitative image analysis program, customized for 16-bit monochrome images generated on a flat-bed scanner. Antibody binding for each lysate was quantified by fitting a negative exponential decay curve plus background and assigned a score based on curve height. These "scores" were then adjusted to account for background variation across the slide, and normalized to total protein. The scores for each antibody-labeled slide were then divided by the standard deviation of spots known to be "empty", as a means of standardizing the information content across antibodies. These results were then entered into a cluster analysis. Hierarchical clustering analysis based on 23 signaling endpoints (antibodies against phosphorylated and non-phosphorylated signaling proteins) classified the tumors into two large groups, one containing 7 of 8 endometrioid tumors in the study set. We expect that profiling the status of signaling pathways in individual ovarian cancer tissues will move us closer to the reality of patient-tailored therapy where appropriate treatments are chosen and monitored based on the proteomic signature of a tumor before, during and after therapy.